On the Making of Genitalia Slides of Lepidoptera

BY AUGUST BUSCK
U. S. Bureau of Entomology and Plant Quarantine, Washington, D. C.

(Presented at the meeting of February 10, 1941)

The value of the study of the genitalia of both sexes in the classification of Lepidoptera is so generally accepted as to require no further arguments. The "fingerprints" are here to stay as an essential part of the study of Lepidoptera (and most other insects); no modern Lepidopterist can afford to neglect them.

The nomenclature of the different parts has been repeatedly given and is happily used reasonably uniformly by Lepidopterists, with only minor variations of some of the terms.[1] Several coworkers, among others your own beloved Otto H. Swezey, have asked for short directions on the technic of making these preparations and this is all that is attempted in the present note.

It may, however, be convenient for the student to recapitulate shortly the terms used for the different parts of both sexes and this may easiest be done by referring to the accompanying figures and the lettering on them.

The male genitalia proper consist of the ninth and tenth abdominal segments and their appendages. In several groups the eighth abdominal segment is also more or less modified and secondarily a part of the genital structure, serving as a covering or exhibiting scale tufts, which undoubtedly play a role in the sexual act.

On the 10th segment the *uncus* (U) may be considered the tergite and the *gnathos* (Gn) the sternite with the *socii* (Si), when present, arising at the base of the uncus; between these terminates the alimentary canal in the anal opening.

The *uncus* is of various form, joined by a membrane to or fused with the tergite of the ninth segment, the *tegumen* (Tg). It may be spoon-shaped or hooklike, smooth or hairy, bifurcate or trifid; in many forms it is much reduced or absent.

The *socii* (Si) are paired organs, arising from the base of the uncus above the gnathos; normally soft and hairy; they may be erect or drooping, cylindrical or flattened; rarely strongly sclerotized; often absent.

The *gnathos* (Gn) is a paired organ, consisting of two arms, arising at the base of the uncus and normally fused at their tips into

[1] The several valuable volumes of illustrations of these structures by F. N. Pierce and Rev. J. W. Metcalfe should be referred to on these subjects. Carl Heinrich's and Busck's short paper on the male genitalia (Proc. Ent. Soc. Wash., Vol. 23, pp. 145-152, pls. 12-13, 1921); Busck: On the Female Genitalia of Microlepidoptera (Bull. Brooklyn Ent. Soc., 26, pp. 199-216, 5 pls., [1931] 1932); as well as W. T. M. Forbes: "Lepidoptera of New York" will also be helpful and accessible to American workers.

a strong hook; but in some families the arms are free and more or less fused with the socii (Sparganotidae). In some families the gnathos is supplanted by a ventral plate (Vp) in the median line below the alimentary canal; the plate may be a broad shield (*Peronea*) or a slender sclerotized rod along the underside of and supporting the alimentary canal (Aegeriidae). The gnathos probably functions as a check on the flow of the content in the alimentary canal; it may be entirely absent (Phalonidae) and its function may then be taken over by a strongly developed *transtilla* (Ts) of the ninth segment.

The ninth segment consists of a large tergite, the *tegumen* (Tg), which forms the back of the genitalia structure and the sternite, the *vinculum* (Vm) of various forms, from a thin ring to a broad shield often with a long pointed prolongation; behind the vinculum is normally found a small, more or less triangular plate, the *anellus* (An) which supports the *aedoeagus* (Ae). Articulated on the vinculum and often also attached to the anellus, are the paired, lateral, more or less wing-shaped clasping organs, the *harpes* (Hp).[2]

The *harpes* are modified pedal appendages of the ninth segment, the joints of which are sometimes defined by actual sutures (Ethmiidae). More often these sclerotized segments are completely fused, but three areas may normally be differentiated by heavier sclerotizations or different armature or scaling, the *costa* (Ca), the *sacculus* (Sc), and the *cucullus* (Cs). These parts are together or separately modified in various ways; the *costa* and the *sacculus* are sometimes developed into free arms, so as to result in double harpes.

The *transtilla* (Ts) in its simplest form is a narrow band connecting the two harpes at their inner costal angles. In some families it is strongly sclerotized and armed at its ends or in its middle with heavy thorns or hooks. In other forms it is attenuated or broken in the middle or reduced to short arms or knobs from the base of the harpes.

The *aedoeagus* (Ae) is a more or less strongly sclerotized tube, which contains and protects the delicate membranous *penis* (P). It may be straight or curved and is often prolonged into one or more spines at the tip and or laterally armed with sharp teeth or other processes. At the base it is commonly bulging out into a blind oval or ball-shaped sack below the entrance hole of the penis. In the higher groups the aedoeagus is normally articulated on the anellus; in the lower it is merely supported by the membrane of the ninth segment, which sometimes forms a tube around it.

Inside the aedoeagus lies the soft flexible *penis* (P), which by blood pressure during copulation is projected far beyond the mouth

[2] The *valvae* of Pierce. Others have used the term clasper for these organs, but that term was first used by J. B. Smith for thorn-like projections from the sacculus, strongly developed in the Noctuidae, with which Smith worked.

of the aedoeagus through the ductus and into the bursa of the female. The penis is often armed with one or several short or long spines, the *cornuti* (Cn) or "love-thorns". These may be permanently attached on the penis or may be deciduous and remain in the bursa of the female after copulation.

In most Lepidoptera the male genital structures are symmetrical, with right and left sides alike, but asymmetry is met with in several groups and is characteristic of entire families (Crambidae, Cosmopterygidae); in such cases there is normally similar asymmetry in the female organs, enabling copulation from only one side.

The female genitalia (in the most numerous main groups of Lepidoptera with two genital openings, one for copulation and one for egg-laying) terminate normally in a pair of flattened hairy lobes, the *ovipositor* (Ov), between which lies the anus and the end of the egg duct. The ovipositor, however, in many Lepidoptera is variously modified, often telescopic for the deposition of the eggs in deep crevices or strongly sclerotized for cutting purposes.

On the underside of the eighth abdominal segment or in the connecting tissue between the eighth and the seventh segment is found the second genital opening, the *ostium* (Os) through which copulation takes place. The ostium is generally surrounded by a more or less defined *genital plate* (G. P.) and leads into the *ductus bursae* (D) which connects it with the *bursa copulatrix* (B) where the male sperma is deposited. The *bursa* is commonly armed on the inside with one or more sclerotized plates or thorns, the *signum* (S), which, when present, offers excellent characters for classification. In many species, however, it is absent. The *ductus* is normally a simple short tube, but in some groups it is very long—sometimes twice as long as the abdomen and therefore bent upon itself or spiraled and in such cases often strengthened by sclerotization.

In the other, more primitive, smaller main group of Lepidoptera with only one sex opening at the tip of the abdomen the *ostium* is lacking but the *ductus* and the *bursa* with or without its *signum* are present.

The technic in the preparation of these sex organs for microscopic study is very simple and can be acquired by anyone with good eyes and nimble fingers with a few hours' practice.

First (1) write your labels, one for the specimen stating merely: ♂ (or ♀) genitalia on slide number ....., date and the maker's initials; the other label—to be eventually copied on the slide when finished—*giving the same data as above and the full date and locality data, collector, host plant, etc., as found on the specimen.* In this way it is always possible to connect the specimen and the slide.

(2) Detach the abdomen. This can normally be done easily by lifting it upwards with a stiff needle; avoid a springy pin, as the

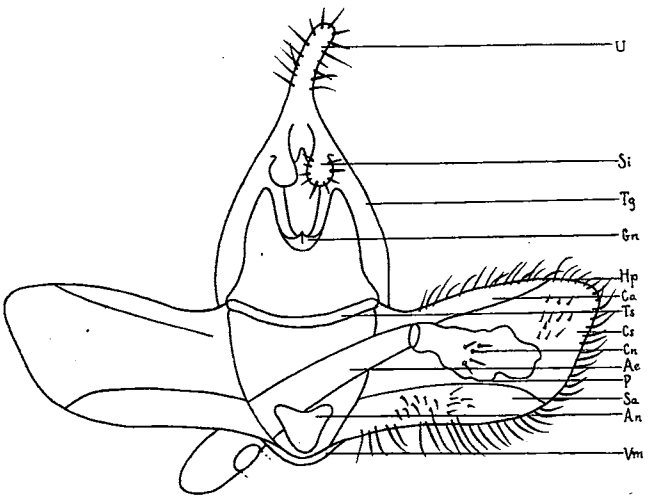

Fig. 1.

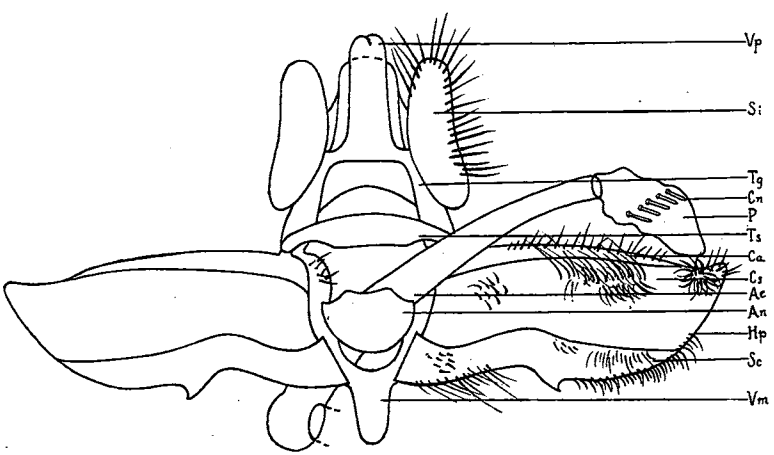

Fig. 2.

Male genitalia of Lepidoptera. Diagrammatic.

abdomen, if small, is apt to jump and get lost. Avoid especially a glued on abdomen, which may or may not truly belong to the specimen and which therefore may lead to very disastrous conclusions.

(3) Drop detached abdomen for a minute in alcohol until saturated.

(4) Drop abdomen into a small vial with a nearly saturated water solution of Potassium Hydroxide (KOH) and pin your temporary label firmly to the cork. Several vials may conveniently be kept upright in a rack.

(5) Leave small abdomens in the cold KOH until the next day; large fatty abdomens may require a longer time.

(6) Place the now soft abdomen in clean water in a small glass or porcelain dish and clean out the inside of the abdomen by *gentle* pressures with a small *soft* camel hair brush.

(7) Place the then empty skin in water on a slide and finish cleaning under the microscope. Manipulate with two fine micro pins firmly stuck into handles, preferably somewhat obliquely. Separate the male genitalia from the rest of the abdomen by inserting one pin just under the edge of the tegumen, holding the rest of the abdomen with the other pin. If the specimen is in the right condition, this is easily accomplished and the genitalia will come off with clean edges. Be careful that the *aedoeagus* comes out with the genitalia; it sometimes has a tendency to be retained in the abdomen. Still using your fine pins in holders endeavor to open the *harpes,* so as to obtain a full face view of the inside of the structure. With most Lepidoptera this can be done easily; but in some families (Gelechiidae) the short *transtilla* or other characteristics of the structure prevent the opening of the *harpes* without breaking or dislocating parts of the structure. In such cases the mount should be made for a lateral view. If the specimen is small, color it (not too strongly!) with a drop of Mercurichrome in the water on the slide.

(8) Dehydrate the abdomen and genitalia thoroughly in 95 per cent alcohol. This hardens and stiffens the genitalia somewhat, but they may still be manipulated carefully with the two micro pins and brought into the desired spread-out position.

(9) Lift genitalia and abdominal skin with your micro pin and place on a slide in a drop of oil of cloves for clearing. Keep the label with the slide. The genitalia may with advantage remain in the oil of cloves for an hour or even a day, but they become brittle and must then be handled with care.

(10) Drop into Xylol to remove the oil; the specimens are then ready to be mounted in balsam. Place on a clean slide, cover with a suitable drop or more of Canada balsam and place the cover glass on it, first wetting the underside with Xylol to avoid bubbles in the

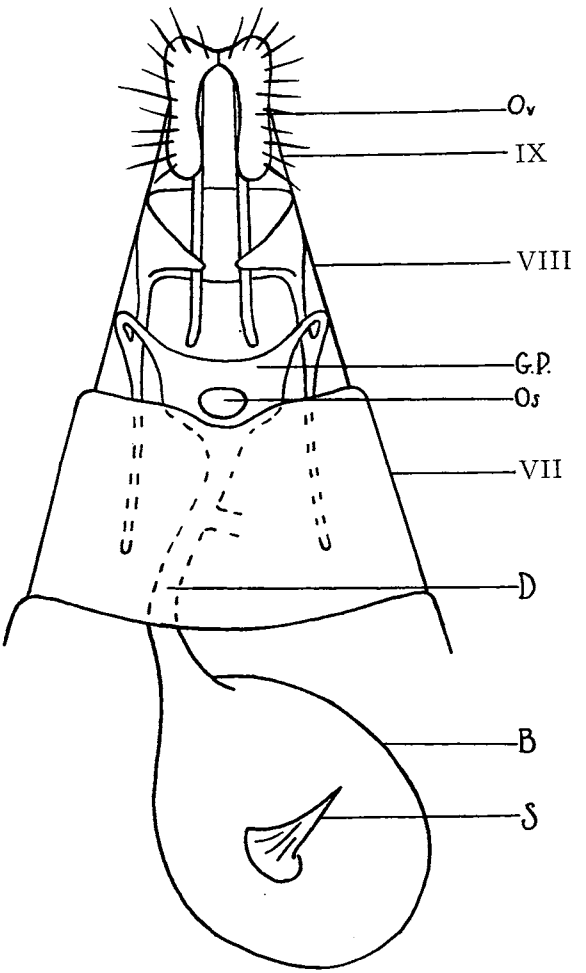

Fig. 3.

Female genitalia of Lepidoptera. Diagrammatic.

balsam. Use a rather thin balsam diluted with Xylol. If the specimen is large, use a drop slide.

(11) *Label—giving all the data.*

(12) The female genitalia require a somewhat different treatment in order to have the internal parts exposed. When they are in the water on a slide as in (7), tear the abdominal skin in two between the seventh and the sixth segments with your micro pins, using care not to cut the *ductus bursae,* pull it and the bursa out intact, connected with the anal segment. Otherwise proceed as with the male.

With large moths and butterflies like the *Sphingidae* where it is desirable to retain the abdomen on the specimen and where the abdominal skin is too large for an ordinary slide, the genitalia can be picked out after the specimen is thoroughly relaxed.

The schematic drawings illustrating these notes were made by Miss Bernice Harper of the Bernice P. Bishop Museum staff and I wish to express my thanks to her for this and other kind help to me during my stay in Honolulu.

EXPLANATION OF FIGURES

FIG. 1. Hypothetical male genitalia of a Lepidopteron with gnathos present.

FIG. 2. Hypothetical male genitalia of Lepidopteron with gnathos absent, supplanted by a ventral plate.

FIG. 3. Hypothetical female genitalia of Lepidopteron with two sex openings.

Figures 1 and 2, male

Ae—aedoeagus
An—anellus
Ca—costa
Cn—cornuti
Cs—cucullus
Gn—gnathos
Hp—harpes
Sc—sacculus
Si—socii
Tg—tegumen
Ts—transtilla
U—uncus
Vm—vinculum
Vp—ventral plate

In figure 3, female

VII—Seventh segment
VIII—Eighth segment
IX—Ninth segment
B—bursa copulatrix
D—ductus bursae
G.P.—genital plate
Os—ostium
Ov—ovipositor
S—signum